Enhancing Water Use Efficiency in Corn

Michael Luethy
Jacqueline Heard
March 5, 2007
Impact of drought on maize yield

Percent yield reduction associated with water deficits (four consecutive days of visible leaf rolling)

5-10%  10-25%  40-50%  30-40%  20-30%


(MONSANTO BIOTECHNOLOGY)
Farmer Needs for More Reliable and Efficient Production Define the Product Concepts and Drive Our Discovery Process

- Irrigated Acres: Protect aquifer water levels / Reduce fuel consumption needed to pump water
- Western Dryland Acres: Yield benefits due to drought trait / Opportunity for more crop choices for growers
- Central Corn Belt: Yield Stability / Consistency

Map showing low and high annual precipitation areas.
Plants activate a diverse set of physiological, biochemical and molecular responses to drought leading to adaptation.

- **Physiological Responses**
  - Decreased Turgor
  - Reduced leaf WP (y)
  - Decreased Stomatal Conductance
  - Reduced Internal CO$_2$
  - Decrease in net PS
  - Osmotic Adjustment
  - Reduced Mitosis
  - Decreased LER

- **Biochemical Response**
  - ABA production & transport
  - Decreased RUBISCO activity
  - Decreased RuBP
  - Decreased SPS, FBPase, PEPcase
  - Production of ROS
  - Accumulation of compatible solutes, amines, sugars, sugar alcohols
  - Increase in Antioxidants
  - Decrease in ROS

- **Molecular Responses**
  - Signal Perception
  - ABA synthesis genes induced
  - ABA responsive genes induced
  - Upregulation of Stress-Protective genes (hsp/Chaperones, LEA, RAB, dehydrins etc)
  - Upregulation of regulatory genes (TFs)
Diverse Gene Families Have Demonstrated Qualitative Drought Tolerance in Transgenic Plants: Models & Crops

Signaling & Transcriptional Control

Membrane protection, osmoprotectants & antioxidants

Signaling Factors

Functional Proteins

Transcription factors

From: Umezawa et al 2006 Current Opinion in Biotechnology 17:113
Drought Tolerant Crops

• Conventional Breeding
• MAS/QTLs
• Plant Biotechnology

* Stress Tolerance Proteins
* Response regulators
* Stress associated metabolites

Lead to:
• Step changes in yield with transgenic trait
• Impact multiple genes / pathways
• Breeding for secondary traits/target market adaptation
Phases of Product Development: identifying product candidates from discovery efforts

<table>
<thead>
<tr>
<th>Phase</th>
<th>Discovery Gene/Trait Identification</th>
<th>Phase I Proof Of Concept</th>
<th>Phase II Early Development</th>
<th>Phase III Advanced Development</th>
<th>Phase IV Pre-launch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Duration¹</td>
<td>24 to 48 MONTHS</td>
<td>12 to 24 MONTHS</td>
<td>12 to 24 MONTHS</td>
<td>12 to 24 MONTHS</td>
<td>12 to 36 MONTHS</td>
</tr>
<tr>
<td>Genes in Testing</td>
<td>TENS OF THOUSANDS</td>
<td>THOUSANDS</td>
<td>10s</td>
<td>&lt;5</td>
<td>1</td>
</tr>
</tbody>
</table>

Key Inflection Point

1. Time estimates are based on our experience; they can overlap. Total development time for any particular product may be shorter or longer than the time estimated here.
Gene selection is primarily driven by model system results

Identify pathways associated with product concepts

Priority

Depth of sampling depends on depth of pipeline, strength of evidence and expected value of product

Identify pathways associated with product concepts

hits

homologues

interacting proteins

coordinate regulation

gene families

Genes with demonstrated over expression phenotypes

Some genes are more likely to produce phenotypes

high

low
Additional Criteria for Gene Selection

- Systematic search to avoid genes with similarity to known allergens or toxins
- IP status reviewed
- Duplicates filtered out

Sequence identified

Cloning

Allergen screen
Toxin screen
IP screen
Duplicate screen
High Throughput Corn Transformation

Thousands of events produced annually
HTP Technologies Support Molecular Analysis

- mRNA Expression Levels
- QC of plants
Multi-season Breeding Process to produce seed for screens and testing

- **R0**
  - **RP**
  - **BC1F1**
    - **Self**
      - **BC1S1**
        - **Self**
          - **BC1S2** (++)
            - **Tester**
              - **Hybrid**

Seed flows from each step to **Archive** and **Early Inbred Evaluation**:
- Seed analytics
- Trait teams

Seed flows from each step to **Inbred/Hybrid Evaluation**:
- approved sites
- Seed analytics
- Trait teams
Identifying Lead Genes for Drought Tolerance Using Functional Genomics Platform

- High Throughput Cloning, Transformation, Greenhouse & Field Screens
  - Detailed Physiology Trials
    - Controlled Drought Field Yield Trials
    - Multiple Location & Germplasm Yield Trials

Broad net → Narrow Field → Top Candidates → Develop Products
Transgenes create variation with respect to yield under drought:

_Yield under Controlled Drought Key Measure for Advancement_

% Change in Yield Under Drought Stress Conditions

Events Screened

Genes represented in this trial had demonstrated GH drought efficacy
Yield and Productivity Remain the Keys to Successful Drought Tolerance Trait

- Gene

+ Gene

Hybrid 1: 2004, 4 locations; 2005 5 locs; 2006, 1 loc (20 reps)
Hybrid 2: 2004, not tested; 2005, 5 locs, 2006, 3 locs
Focus on Traits Associated with Yield Under Drought

Chlorophyll content
Leaf rolling
ASI = SD - AD
Tassel size
Growth
Senescence, stay-green
Leaf firing, tassel blasting

Additional Traits: Grain Set, Grain Fill, Kernel Abortion, etc.

Betran, 2005
Consistent replication of drought stress is the key to finding new genes.
Reiterative Field Testing under Target Water Stress, Multiple Environmental Data Points To Identify Lead Lines for Advancement

Corn Drought Yield Testing Network

- Natural rainfall or irrigated
- Controlled drought stress
- Controlled drought & full irrigation

Multiple Locations

Multiple Replications per Location

Aerial photo of leaf temperature

- Stressed
- Watered

Multiple Plant & Environment Measurements
Associated traits important for drought tolerance

- Ideal **Secondary Traits** need to be highly correlated to yield under stress, carry no yield penalty under favorable conditions, be highly heritable, cheap and easy to measure, stable over time, and be observed at or prior to flowering.
- Corn grain yield is most sensitive to water and heat stress from just before silking through grain fill.

**Key secondary focus traits:**
- Protogyny or a negative anthesis-silking interval (ASI)
- Increased prolificacy (reduced barrenness)
- Improved staygreen
- Reduced tassel size
- Larger ears prior to anthesis
- Rooting depth and structure
- Shorter plants at flowering
- Upright leaf habit

**Disease concerns:**
- Resistance to *Aspergillus flavus* infection and aflotoxin contamination – high temperatures and drought have been reported to increase growth of the fungus and toxin production (Payne, 1998)
- Resistance to Goss’s Wilt
Product development testing: Research conducted to advance discovery leads to commercial traits

- Multiple-Year Field Trials:
  - Progression in numbers of environments
  - Regulated by USDA/APHIS
  - Germplasm combining ability

- Evaluate stacks with Existing Commercial Traits and Germplasm

- Conduct Regulatory Science studies required to support applications to appropriate agencies.

- Integrate traits into elite germplasm and existing commercial stacks at time of launch.
Drought Tolerant Corn – Summary

➢ A discovery and development pipeline established:
  ▪ A pipeline for traits that improves yield by improving water utilization efficiency

➢ Making progress:
  ▪ Multiple genes provide degrees of yield stability / drought stress tolerance.

➢ Now - intensive testing:
  ▪ more environments, assessing stacked with existing traits in best adapted germplasm - test fit to each market segment; and commercial event selection.
Thank You